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Effects of Some Representative Petroleum Refinery Effluent Compounds on Photosynthesis and Growth of Natural Marine Phytoplankton Assemblages. Part 1. Cresols

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16. ABSTRACT

The effects of cresols on the photosynthesis and growth of natural marine phytoplankton assemblages from the Scripps Pier at La Jolla, CA were measured. In two experiments, in which dinoflagellates were the dominant algae, photosynthesis (measured by ^{14}C uptake) was inhibited at concentrations greater than 100 g $litre^{-1}$ and the concentration giving half-maximal photosynthesis was 10000 g $litre^{-1}$. In a third experiment, in which diatoms were dominant, photosynthesis was not inhibited until levels greater than 10000 g $litre^{-1}$ were reached and the half-maximal concentration was 20000 g $litre^{-1}$. These experiments suggested that dinoflagellates were more sensitive to cresols than diatoms. However, the reverse was true in an experiment using cultures. In a long-term growth experiment (natural assemblages from the Scripps Pier), only a level of 88,000 g $litre^{-1}$ completely inhibited growth. There was a two-day lag in growth at 8500 g $litre^{-1}$ but the algae recovered and grew as well as the controls which contained no cresols. This experiment was started with a mixed diatom-dinoflagellate-microflagellate population but only the diatoms grew. High concentrations of cresols (greater than 900 g $litre^{-1}$) resulted in a decrease in the diatom Skeletonema costatum, compared with the controls. Otherwise there was no selection for resistant species. In these experiments, cresols were measured by extraction with methylene chloride from seawater followed by UV absorption. In all experiments cresol levels decreased with time, attributed to uptake by phytoplankton & bacterial degradation.

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EFFECTS OF SOME REPRESENTATIVE PETROLEUM REFINERY EFFLUENT COMPOUNDS ON PHOTOSYNTHESIS AND GROWTH OF NATURAL MARINE PHYTOPLANKTON ASSEMBLAGES: PART 1—CRESOLS†

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ABSTRACT

The effects of cresols on the photosynthesis and growth of natural marine phytoplankton assemblages from the Scripps Pier at La Jolla, California, USA were measured. In two experiments, in which dinoflagellates were the dominant algae, photosynthesis (measured by ^{14}C uptake) was inhibited at concentrations $> 100\ \mu g\ litre^{-1}$ and the concentration giving half-maximal photosynthesis was $10{,}000\ \mu g\ litre^{-1}$. In a third experiment, in which diatoms were dominant, photosynthesis was not inhibited until levels $> 10{,}000\ \mu g\ litre^{-1}$ were reached and the half-maximal concentration was $20{,}000\ \mu g\ litre^{-1}$. These experiments suggested that dinoflagellates were more sensitive to cresols than diatoms. However, the reverse was true in an experiment using cultures. In a long-term growth experiment (natural assemblages from the Scripps Pier), only a level of $88{,}000\ \mu g\ litre^{-1}$ completely inhibited growth. There was a two-day lag in growth at $8500\ \mu g\ litre^{-1}$, but the algae recovered and grew as well as the controls which contained no cresols. This experiment was started with a mixed diatom–dinoflagellate–microflagellate population but only the diatoms grew. High concentrations of cresols ($> 900\ \mu g\ litre^{-1}$) resulted in a decrease in the diatom Skeletonema costatum, *compared with the controls. Otherwise there was no selection for resistant species. In these experiments, cresols were measured by extraction with methylene chloride from seawater followed by measurement by UV absorption. In all experiments cresol levels decreased with time. This was attributed to uptake by phytoplankton and bacterial degradation.*

† Contribution from the Scripps Institution of Oceanography.

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INTRODUCTION

Petroleum refineries often discharge their effluents into coastal or estuarine waters. These effluents carry a number of organic compounds which can have various effects on the marine biota. Our main purpose was to ascertain the effects of compounds on the marine phytoplankton. Since these organisms provide, through photosynthesis, most of the organic matter on which other marine organisms (such as zooplankton, larval fish and shellfish) feed, the importance of the phytoplankton to the marine food web is unquestioned. If, for example, the phytoplankton algae were inhibited by compounds in refinery effluents, important fisheries might decline. On the other hand, if concentrations of effluent compounds were found to be relatively noninhibitory to phytoplankton, it might not be necessary to spend large sums to abate the discharge of these compounds.

One class of compound discharged from refineries is the cresols. Buikema *et al.* (1979) have recently reviewed the literature on phenolic compounds in aquatic ecosystems. They list *o*-cresol concentrations in three refinery effluents at 0·12, 0·[illegible] and 5842 mg litre^{-1}. Raphaelian & Harrison (1978) report a concentration of cresols of 50 μg litre^{-1} in a refinery effluent that had been treated by dissolved air flotation and this could be nearly completely removed by activated charcoal or activated sludge treatment. *O*-cresol at 56 mg litre^{-1} inhibits cyclosis in the alga *Nitella* (Buikema *et al.*, 1979). Otherwise these authors list no other work on the effect of cresols on algae. The related compound, phenol, inhibited *Chlorella* at 40 mg litre^{-1}; chlorinated phenolic compounds were somewhat more inhibitory. Thirumurthi & Gloyna (1965) and Huang & Gloyna (1967) studied the toxicity of cresols to *Chlorella*. They report that 250–800 mg litre^{-1} inhibited chlorophyll formation by this freshwater alga. There were no significant differences in the relative toxicity of the three cresol isomers to *Chlorella*.

The present paper describes the effects of a mixture of the three cresol isomers on natural marine phytoplankton assemblages and cultures taken from the Scripps Pier. Four experiments were performed on phytoplankton photosynthesis and one experiment was completed on the effects of cresols on growth and species selection. The work is unique in that, except for one experiment, natural populations, rather than cultures, were used.

MATERIALS AND METHODS

For photosynthesis experiments, a sample of surface seawater containing a natural marine phytoplankton assemblage was collected on three occasions—3 January, 1979, 1 February, 1979 and 14 March, 1979—from the Scripps Pier. The seawater was filtered through 200 μm mesh netting to remove large zooplankton and was then dispensed in 100-ml subsamples into 125-ml bottles which were glass-stoppered after filling.

A cresol mixture (equal parts of the three isomers) was added to the bottles to give the following nominal concentrations: 0, 1, 5, 10, 50, 100, 500, 1000, 5000, 10,000, 50,000 and 100,000 μg litre^{-1} in five sets of twelve bottles. One set was placed in a refrigerator for later cresol analysis. The other four sets were placed in a lighted water bath and incubated at 15 °C. A light intensity of 200 μeinsteins m^{-2} s^{-1} and a 12:12 light–dark cycle was used with these sets.

Incubation was carried out for 48 h. At that time another set was placed in the refrigerator for later cresol analysis and 5 μCi $Na_2{}^{14}CO_3$ solution (specific activity, 8·4 mc/mmole) was added to the other three sets for photosynthesis measurements. One set was darkened with aluminium foil and it and the other two sets (light bottles) were incubated for a further four hours in the water bath. The ^{14}C labelled phytoplankton were then filtered onto 0·45 μm membrane filters which were washed with filtered seawater to remove any residual radioactive carbonate solution. The wet filters were then placed in scintillation vials to which 10 ml Aquasol liquid scintillation fluid was added. The radioactivity on each filter was measured with a Beckman LSC-100 liquid scintillation counter. Quenching was measured using the external standard method and did not vary with the concentration of cresols. The results are reported as cpm (light minus dark) versus analysed initial concentration of cresols.

To compare the effects of cresols on the photosynthesis of two taxonomic classes of phytoplankton—diatoms and dinoflagellates—exponentially growing cultures were used. The diatoms were *Asterionella glacialis* and *Chaetoceros affinis*; the dinoflagellates were *Gonyaulax polyedra* and *Gymnodinium splendens*. Scripps Pier seawater was membrane filtered and 90 ml were placed in 125-ml bottles. To one set of these 5 ml of each diatom culture were added; to another set 5 ml of each dinoflagellate culture were added. Cresols were then added at the concentrations as described above and the bottles were incubated for Photosynthesis as a function of concentration of cresols was then measured as described above for natural population experiments.

For experiments on growth and species selection a seawater sample containing natural phytoplankton, collected on 24 April, 1979, was dispensed in 3·5-litre amounts into 4-litre glass bottles. Twenty bottles were used; ten contained added nutrients at the *f*/10 concentration (Guillard & Ryther, 1962) to enhance algal growth and ten contained no added nutrients. Cresols were added at the following nominal concentrations: 0, 100, 1000, 10,000 and 100,000 μg litre^{-1}. The bottles were sampled initially for *in vivo* fluorescence (a measure of chlorophyll—Lorenzen, 1966; Thomas *et al.*, 1974) and at daily intervals thereafter. Every other day samples were taken for cresol analysis; subsamples were preserved with Lugol's I_2 solution for microscopic identification, counting of the cells and conversion to phytoplankton carbon (Thomas & Seibert, 1977). The bottles were incubated for 10 days in an outdoor incubator using natural light (attenuated with a cotton sheet to give 30 % of full sunlight) and running seawater with a temperature of 18 °C for cooling.

Concentrations of cresols (a mixture of equal parts *m*-, *o*- and *p*-cresol isomers) in the seawater contained in the experimental bottles were determined by ultraviolet spectrophotometry. Cresols were extracted from 100-ml filtered (0·45 μm pore size) seawater samples with two 5-ml volumes of methylene chloride, following acidification with 0·1 ml concentrated HCl. Acidification aided in phase separation of the solvent/seawater system. Methylene chloride was chosen over other solvents (hexane, chloroform and ethers) because of its superior extraction efficiency (*ca.* 95%). Cresols were determined in the extracts by comparing absorbance at 272 nm with that of standard mixtures. The 272 nm absorbance peak was chosen because it was a feature common to all three isomers. A Beckman ACTA M-series recording spectrophotometer was used which provided a limit of detection of 0·1 μg litre^{-1}. The replication of this procedure in the concentration range 10 μg litre^{-1} to 10 mg litre^{-1} was ±5%. Absorbance spectra of experimental extracts deviated only slightly from those of cresol mixture standards; this suggests that there was little interference by extraneous UV-absorbing material in seawater. In this work purified cresols were used; they were purified by double distillation in glass.

RESULTS

Photosynthesis (light minus dark cpm) of natural assemblages as a function of initial cresol concentration is shown in Figs 1 and 2. In the first experiment (Fig. 1), inhibition of photosynthesis started at about 100 μg litre^{-1} and the concentration showing a half-maximal value for ^{14}C uptake was about 10,000 μg litre^{-1}.

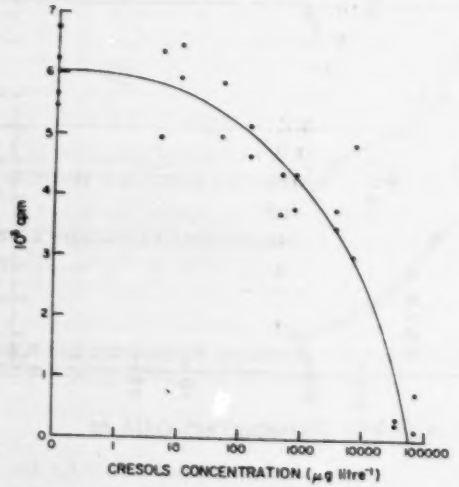

Fig. 1. Photosynthesis (light minus dark cpm) in natural assemblages of marine phytoplankton as a function of analysed cresols concentrations. Experiment 1. Dinoflagellate dominance. 3 January, 1979.

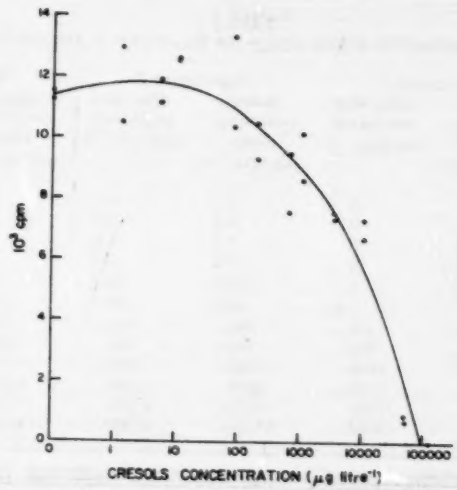

Fig. 2. Photosynthesis (light minus dark cpm) in natural assemblages of marine phytoplankton as a function of analysed cresols concentrations. Experiment 3. Dinoflagellate dominance. 14 March, 1979.

Inhibition was nearly complete at the two highest concentrations. The initial community consisted mainly of thecate dinoflagellates and small (2–4 μm) flagellates.

In a second experiment there was considerable scatter in the data at concentrations below 10,000 μg litre^{-1} and inhibition was only found at the two highest concentrations—44,000 and 87,500 μg litre^{-1}. The concentration showing a half-maximal value for photosynthesis would be about 20,000 μg litre^{-1}. In this case the community was dominated by diatoms.

The results of the third experiment (Fig. 2) were similar to those of experiment 1. Again, inhibition started at about 100 μg litre^{-1} and the concentration giving half-maximal photosynthesis was about 10,000 μg litre^{-1}. In this experiment the community was again dominated by dinoflagellates.

Table 1 gives the analysed initial cresol concentrations and the final concentrations for each of the above photosynthesis experiments. There were considerable losses of cresols at the lower concentrations but not at concentrations >100 μg litre^{-1}.

Comparison of the three photosynthesis experiments in which natural communities were used suggested that diatoms (experiment 2) were less sensitive to cresols than dinoflagellates (experiments 1 and 3). To test this hypothesis we measured photosynthesis in cultures of each taxonomic group. The results are shown in Fig. 3. Contrary to the hypothesis, diatoms were more sensitive than dinoflagellates. The half-maximal value for diatom photosynthesis was about

TABLE 1

ANALYSED CRESOL CONCENTRATIONS BEFORE AND AFTER INCUBATION IN PHOTOSYNTHESIS EXPERIMENTS

Nominal concentrations ($\mu g\ litre^{-1}$)	*Experiment 1*		*Experiment 2*		*Experiment 3*	
	Initial concentrations ($\mu g\ litre^{-1}$)	*After 48 h incubation* ($\mu g\ litre^{-1}$)	*Initial concentrations* ($\mu g\ litre^{-1}$)	*After 48 h incubation* ($\mu g\ litre^{-1}$)	*Initial concentrations* ($\mu g\ litre^{-1}$)	*After 48 h incubation* ($\mu g\ litre^{-1}$)
0	0	0	0	0	0	0
1	0	0	0·6	0·2	1·4	0·1
5	5·0	0	2·5	1·8	6·0	1·4
10	9·6	0	7·4	1·6	11·7	3·8
50	46·2	0	58·9	26·2	93·1	35·4
100	132	1·0	88·0	87·7	210	159
500	461	414	440	440	695	444
1000	760	830	880	880	1080	810
5000	3550	1600	3040	2990	3750	3310
10000	6660	5300	8800	8800	10300	10160
50000	35100	37900	43970	43970	51880	43380
100000	71100	73800	87520	87960	126900	136480

5000 μg litre^{-1}. For dinoflagellate photosynthesis only the two highest concentrations were inhibitory and the half-maximal concentration was about 50,000 μg litre^{-1}. The phytoplankton cultures were isolated from Scripps Pier water.

The effects of concentration of cresols on the growth of a natural community are shown in Fig. 4. Only the fluorescence in bottles containing nutrients is shown

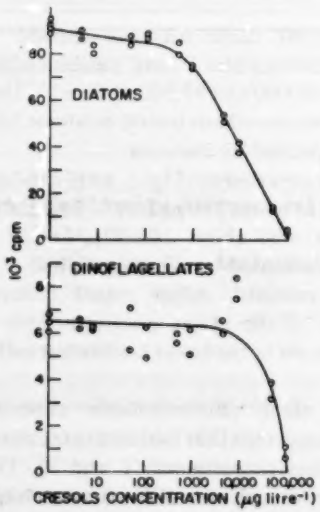

Fig. 3. Photosynthesis (light cpm only) in cultures of diatoms and dinoflagellates as a function of analysed cresols concentration.

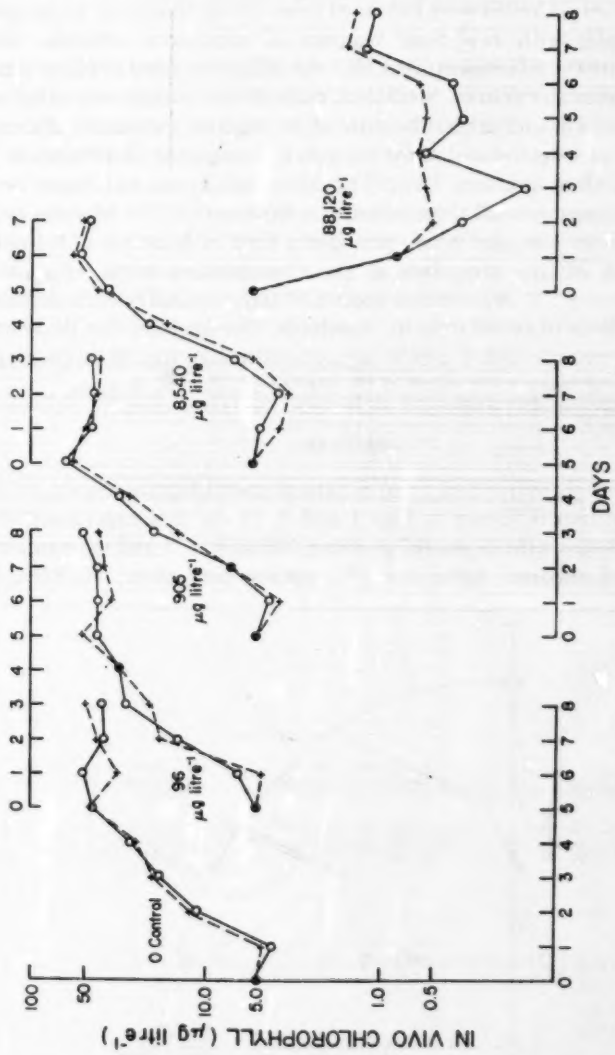

Fig. 4. Growth of natural marine phytoplankton assemblages at four cresols concentrations. The growth curves are displaced for clarity. The + and 0 points represent replicates taken from separate containers.

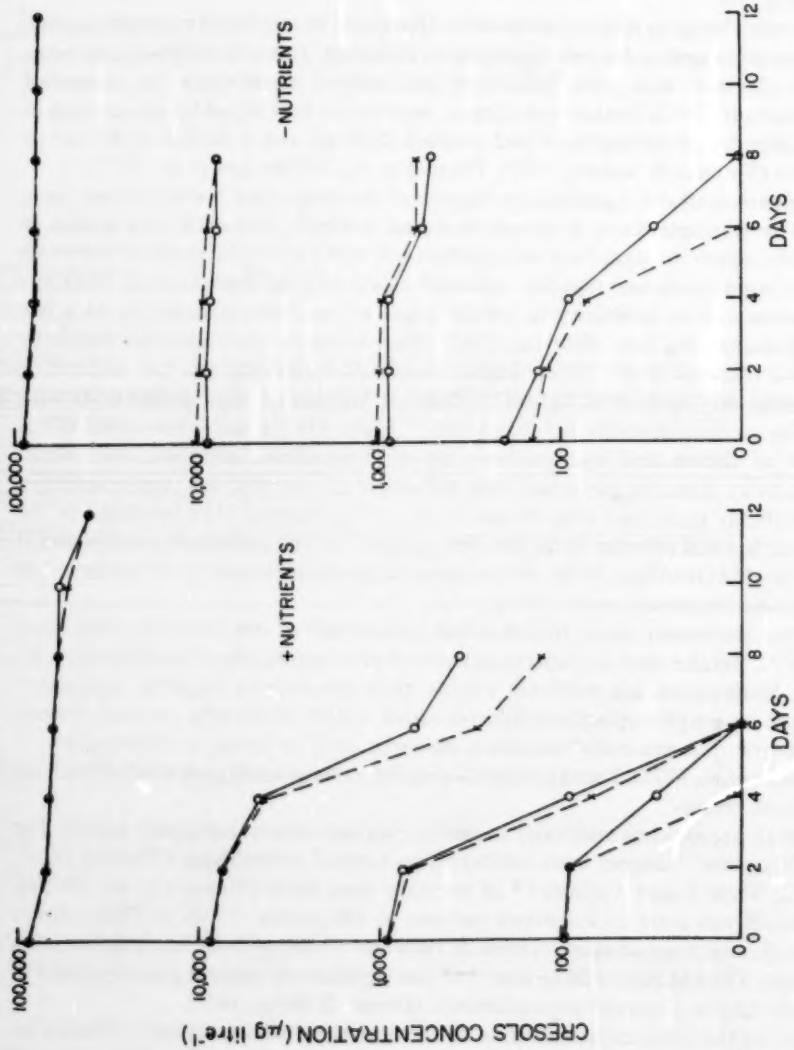

Fig. 5. Changes in cresols concentrations during growth of natural marine phytoplankton assemblages in the presence and absence of nutrients.

because the other bottles gave no growth. Concentrations of 96 and 905 μg litre^{-1} (initial analysed values) had no effect. In the control, there was a lag of one day, followed by exponential growth and senescence. A concentration of 8540 μg litre^{-1} initially inhibited growth so that the lag period was two days; thereafter exponential growth occurred. A concentration of 88,120 μg litre^{-1} completely inhibited growth.

The concentrations of cresols in each treatment during this experiment are shown in Fig. 5. When growth occurred (in treatments with nutrients) there was a marked decrease in concentrations with time, especially at the lower nominal concentrations. Without nutrients this decrease was minimal except at a nominal concentration of 100 μg litre^{-1}.

The initial phytoplankton assemblage consisted mainly of the centric diatoms, *Chaetoceros* spp., the pennate diatom, *Nitzschia delicatissima*, two dinoflagellates —*Ceratium furca* and *Prorocentrum micans*—and small (2–4 μm) unidentified flagellates. After exponential growth had occurred (four-day samples) in treatments containing nutrients, the assemblage in the control cultures (*O*-cresols) consisted mainly of the centrics, *Chaetoceros* spp., and *Skeletonema costatum*, the pennate diatom, *N. delicatissima*, and small unidentified flagellates. The population in the

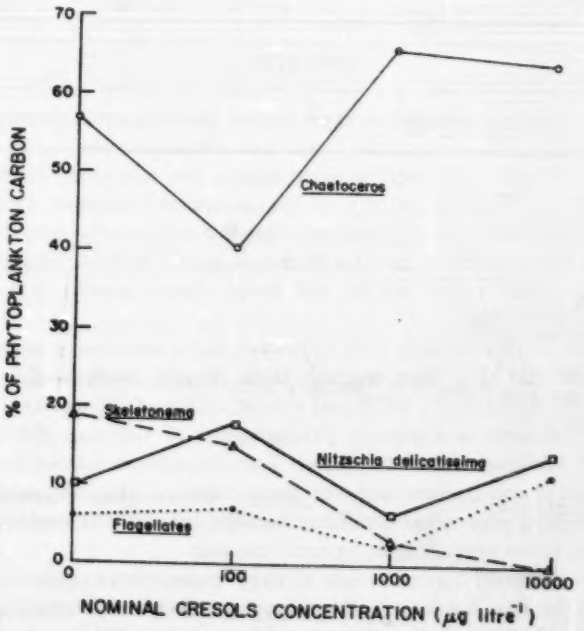

Fig. 6. Percent composition of the biomass of dominant phytoplankton during the exponential phase of growth as a function of nominal cresols concentrations.

treatments having an initial nominal cresol concentration of 100 μg litre^{-1} was similar; at 1000 μg litre^{-1} *Skeletonema* and flagellate numbers were reduced. When logarithmic growth had occurred (six-day samples) in the treatment having an initial nominal concentration of 10,000 μg litre^{-1}, the community was similar to that of the other treatments except that *Skeletonema costatum* was completely absent. It was not possible to examine samples containing 100,000 μg litre^{-1} cresols because a precipitate occurred when the Lugol's I_2 preservative was added to these samples. Note that no growth occurred at this concentration. Figure 6 shows the percent composition of the biomass in terms of carbon during exponential growth as a function of nominal cresol concentration. As a result of cresol addition, no one species or group was predominant over another with the exception that *Skeletonema* was greatly reduced with increasing concentration and was absent at 10,000 μg litre^{-1}.

The inverted microscope technique used for counting phytoplankton is not a quantitative technique for counting bacteria. However, qualitative changes in bacteria could be seen. In samples containing 100 or 1000 μg litre^{-1} cresols, few bacteria were seen. At a concentration of 10,000 μg litre^{-1} there were many bacteria during exponential phytoplankton growth.

DISCUSSION

In both photosynthesis and growth experiments, the only concentration of cresols that was strongly and consistently toxic was 100,000 μg litre^{-1}. In the photosynthesis experiments with natural assemblages, the inhibitory level seemed to depend on the species composition of the community samples. Dinoflagellates seemed to be more sensitive than diatoms, but this was not the case with cultures. Ten thousand microgrammes per litre of cresols gave a half-maximal inhibition in photosynthesis experiments but in the longer term growth experiment this inhibition was overcome.

These marine phytoplankton were somewhat more sensitive to cresols than the few other algae that have been studied. With *Nitella*, cyclosis was inhibited at 56 mg litre^{-1} (Buikema *et al.*, 1979) and it took a dose of 250 mg litre^{-1} to inhibit chlorophyll formation in *Chlorella* (Thirumurthi & Gloyna, 1965; Huang & Gloyna, 1967). However, the studies using *Chlorella* were carried out in the presence of high nutrient concentrations and with dense cultures. The comparison with our results using marine microalgae is difficult because our nutrient levels and densities were more like those prevailing in natural seawater.

The decline of cresol concentration in these experiments could be due to (1) absorption by the glass bottles, (2) absorption by non-living particulate matter, (3) uptake by phytoplankton and/or (4) bacterial degradation. We have no evidence for hypotheses (1) or (2). Uptake by phytoplankton or bacterial degradation may have occurred since the rate of decrease in cresol concentration was faster in the presence of nutrients than in their absence and bacteria increased at a cresol concentration of 10,000 μg litre^{-1}.

The only change in species composition that could be attributed to cresols was the decrease in the centric diatom, *Skeletonema costatum*. This is in contrast to previous results obtained with other pollutants and natural assemblages. In controlled ecosystems (CEPEX containers) copper, mercury or fuel oil additions resulted in dominance by microflagellates and pennate diatoms and a decline in all centric diatoms (Thomas & Seibert, 1977; Thomas *et al.*, 1977*b*; Lee *et al.*, 1977).

Compared with other potential pollutants of the ocean, cresols were not very toxic to marine phytoplankton. It should be noted, however, that most such studies of pollutant effects on algae have been carried out with pure cultures rather than with natural populations and that the responses varied with the species tested. DDT has been shown to be inhibitory to certain algae at concentrations as low as a few microgrammes per litre (Wurster, 1968). *Thalassiosira pseudonana* was especially sensitive (Menzel *et al.*, 1970), whilst *Dunaliella tertiolecta* was not affected at concentrations up to 1000 μg litre^{-1}. Natural Vineyard Sound populations were sensitive at concentrations of 5–10 μg litre^{-1}. Fisher (1975), in a reassessment of the effects of chlorinated hydrocarbons on photosynthesis, suggests that overall productivity of marine phytoplankton will not be affected but that species selection will probably occur (see also Mosser *et al.*, 1972). Natural photosynthesis in the Adriatic Sea was affected by 50 and 100 μg litre^{-1} of the polychlorinated biphenyl Aroclor 1254 (Harding, 1976). A blue–green alga was inhibited by 500 μg litre^{-1} of *p*-toluidine (Batterton *et al.*, 1978).

In two freshwater algae, hydrocarbon compounds of low solubility were more toxic (^{14}C uptake used as a test) than those of greater solubility (Hutchinson *et al.*, 1979). Since cresols are relatively soluble, their low toxicity might be expected.

Three marine phytoplankton cultures varied in their response to benzene, toluene and xylene, but generally inhibition occurred only at levels >10,000 μg litre^{-1} (Dunstan *et al.*, 1975). The experiments with these compounds gave similar results to ours with cresols.

Cresols are also less inhibitory to marine phytoplankton than heavy metals. Ten and 50 μg litre^{-1} copper were inhibitory to natural assemblages (Thomas *et al.*, 1977*a*), while 1 and 5 μg litre^{-1} of mercury were toxic (Thomas *et al.*, 1977*b*). Cadmium was toxic to *Isochrysis galbana* at 100 μg litre^{-1} and to *Thalassiosira fluviatilis* and *Amphidinium carterae* at 1000 and 10,000 μg litre^{-1}, respectively (Li, in prep.). The addition of 20 μg litre^{-1} of zinc significantly reduced photosynthesis in natural English Channel phytoplankton (Davies & Sleep, 1979).

Two of the three concentrations of *o*-cresol reported for refinery effluents by Buikema *et al.* (1979) are 120 and 750 μg litre^{-1}. In these cases the effluents would not be toxic with respect to cresol concentration, since the inhibitory levels found in our experiments were around 10,000 μg litre^{-1}. The other refinery reported

5,842,000 μg litre^{-1} in the effluent. In this case a 500:1 dilution by the receiving water would be necessary to reduce the cresol level to an acceptable limit. Cresols could also be removed almost completely by dissolved air flotation (DAF) followed by activated charcoal or activated sludge treatment (Raphaelian & Harrison, 1978) and even the first process (DAF) would reduce the cresols to an acceptable concentration (50 μg litre^{-1}).

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REFERENCES

BATTERTON, J., WINTERS, K. & VAN BAALEN, C. (1978). Anilines: Selective toxicity to blue-green algae. *Science*, **199**, 1068–70.

BUIKEMA, JR, A. L., MCGINNIS, M. J. & CAIRNS, JR, J. (1979). Phenolics in aquatic ecosystems: A selected review of recent literature. *Mar. Environ. Res.*, **2**, 87–181.

DAVIES, A. G. & SLEEP, J. A. (1979). Photosynthesis in some British coastal waters may be inhibited by zinc pollution. *Nature*, **277**, 292–3.

DUNSTAN, W. M., ATKINSON, L. P. & NATOLI, J. (1975). Stimulation and inhibition of phytoplankton growth by low molecular weight hydrocarbons. *Mar. Biol.*, **31**, 305–10.

FISHER, N. S. (1975). Chlorinated hydrocarbon pollutants and photosynthesis of marine phytoplankton: A reassessment. *Science*, **189**, 463–4.

GUILLARD, R. R. L. & RYTHER, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonu'a confervacea* (Cleve) Gran. *Can. J. Microbiol.*, **8**, 229–39.

HARDING, JR, L. W. (1976). Polychlorinated biphenyl inhibition of marine phytoplankton photosynthesis in the northern Adriatic Sea. *Bull. Environ. Cont. & Toxicol.*, **16**, 559–66.

HUANG, J.-C. & GLOYNA, E. F. (1967). *Effects of toxic organics on photosynthetic reoxygenation*. Report to the Federal Water Pollution Control Administration Contract WP-00688-03. Univ. of Texas Center for Research in Water Resources.

HUTCHINSON, T. C., HELLEBUST, J. A., MACKAY, D., TARM, D. & KAUSS, P. (1979). Relationship of hydrocarbon solubility to toxicity in algae and cellular membrane effects. *Proc. Oil Spill Conf. (Prevention Behaviour, Control, Cleanup)*, American Petroleum Institute Publication No. 4308, Washington, DC, 541–7.

LEE, R. F., TAKAHASHI, M., BEERS, J. R., THOMAS, W. H., SEIBERT, D. L. R., KOELLER, P. & GREEN, D. R. (1977). Controlled ecosystems: Their use in the study of the effects of petroleum hydrocarbons on plankton. In: *Physiological responses of marine biota to pollutants*, (Vernberg, F. J., Calabrese, A., Thurberg, F. P. & Vernberg, W. B. (Eds)), Academic Press, New York, 323–41.

LI, W. K. W. Cadmium-stressed growth of unialgal and bialgal cultures. *Mar. Environ. Res.* (in prep.).

LORENZEN, C. J. (1966). A method for the continuous measurement of *in vivo* chlorophyll concentration. *Deep-Sea Res.*, **13**, 223–7.

MENZEL, D. W., ANDERSON, J. & RANDTKE, A. (1970). Marine phytoplankton vary in their response to chlorinated hydrocarbons. *Science*, **167**, 1724–6.

MOSSER, J. L., FISHER, N. S. & WURSTER, C. F. (1972). Polychlorinated biphenyls and DDT alter species composition in mixed cultures of algae. *Science*, **176**, 533–5.

RAPHAELIAN, L. A. & HARRISON, W. (1978). *Trace organics variation across the wastewater treatment system of a Class-B refinery and estimate of removal of refractory organics by add-on mixed-media filtration and granular acticated carbon on a pilot scale*. Report to US Environmental Protection Agency and US Department of Energy, ANL/WR-78-2, EPA-600/7-78-125. Argonne National Laboratory, Argonne, Illinois.

THIRUMURTHI, D. & GLOYNA, E. F. (1965). *Relative toxicity of organics to* Chlorella pyrenoidosa. Report to the US Public Health Service, Contract WP-00688-01A1. Univ. of Texas Center for Research in Water Resources.

THOMAS, W. H., SEIBERT, D. L. R. & DODSON, A. N. (1974). Phytoplankton enrichment experiments and bioassays in natural coastal sea water and in sewage outfall receiving waters off Southern California. *Estuarine & Coastal Mar. Sci.*, **2**, 191–206.

THOMAS, W. H. & SEIBERT, D. L. R. (1977). Effects of copper on the dominance and the diversity of algae: Controlled ecosystem pollution experiment. *Bull. Mar. Sci.*, **27**, 23–33.

THOMAS, W. H., HOLM-HANSEN, O., SEIBERT, D. L. R., AZAM, F., HODSON, R. & TAKAHASHI, M. (1977*a*). Effects of copper on phytoplankton standing crop and productivity: Controlled ecosystem pollution experiment. *Bull. Mar. Sci.*, **27**, 34–43.

THOMAS, W. H., SEIBERT, D. L. R. & TAKAHASHI, M. (1977*b*). Controlled ecosystem pollution experiment: Effect of mercury on enclosed water columns. III. Phytoplankton population dynamics and production. *Mar. Sci. Comm.*, **3**, 331–54.

WURSTER, JR, C. F. (1968). DDT reduces photosynthesis by marine phytoplankton. *Science*, **159**, 1474–5.